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# *Hydnotrya laojunshanensis* sp. nov. from China

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Abstract — In August 2012, a solitary *Hydnotrya* specimen was collected under *Abies forrestii* var. *smithii* in the Laojun mountains, Yunnan Province, southwest China. Morphological and molecular analyses showed that this was a new species, which is described here as *H. laojunshanensis*.

Key words — *Pezizomycetes*, taxonomy, ITS, phylogeny

## Introduction

*Hydnotrya* Berk. & Broome (*Pezizomycetes, Ascomycota*) is a genus of hypogeous fungi related to truffles. The genus was placed in *Helvellaceae* by Spooner (1992), but recent molecular analyses support its placement in the *Discinaceae* (O'Donnell et al. 1997; Hansen & Pfister 2006; Tedersoo et al. 2006; Læssøe & Hansen 2007). *Hydnotrya* is distributed across the northern hemisphere including Europe, North America, and Asia (Stielow et al. 2010). There are about 15 species in the genus (Kirk et al. 2008: 325) including a neglected new species, *Hydnotrya bailii* Soehner (Soehner 1959; Stielow et al. 2010). Four *Hydnotrya* species have been reported in China: *H. cerebriformis* Harkn. in Shanxi and Xinjiang Provinces, *H. cubispora* (E.A. Bessey & B.E. Thomps.) Gilkey in Sejila mountains, Linzhi, Tibet, and *H. michaelis* (E. Fisch.) Trappe and *H. tulasnei* (Berk.) Berk. & Broome in Jilin Province (Tao & Liu 1989; Xu 2000). In August 2012 one *Hydnotrya* specimen was collected in the Laojun mountains, Yunnan Province, southwest China (Plate 1). Morphological examination and DNA sequence analyses clearly distinguished the specimen from all other *Hydnotrya* taxa. We describe it as a new species and discuss its relationships with other *Hydnotrya* species.

Plate 1. Location of Laojun mountains, Yunnan Province, southwest China.

## Materials & methods

Microscopic and macroscopic descriptions were made of specimen YAAS L2425 following the methods of Bemmann & Bandini (2011). Sections were made with a razorblade by hand, mounted in 5% KOH solution or water, and then stained with cotton blue or lactophenol solution. The sections were observed under an Olympus BH-2 microscope. The type specimen was deposited in the Herbarium of Yunnan Academy of Agricultural Sciences, Kunming, China (YAAS).

Total genomic DNA was extracted using the Zomanbio Plant Genomic DNA Kit. The internal transcribed spacer (ITS) rDNA region was amplified with PCR primers ITS4 and ITS5. The PCR reactions were run on a BIO-RAD C1000TM Thermal Cycles with the following settings: initial denaturation for 5 min at 94°C, followed by 32 cycles of 40 s denaturation at 94°C, annealing at 56°C for 40 s, extension for 1 min at 72°C, and final extension at 72°C for 10 min. Purifying and sequencing of PCR products were conducted by Beijing Genomics Institute.

The *Hydnotrya* YAAS L2425 sequence was analyzed molecularly with 35 retrieved from GenBank were used for phylogenetic analysis. *Gyromitra infula* (Schaeff.) Quél. was selected as the outgroup. Sequences were edited and assembled using SeqMan II (Larsson & Sundberg 2011). Alignment of nucleotide sequences was performed by Mafft-win. Sequences were adjusted manually using BioEdit 7.0.1. Phylogenetic analyses were carried out via PAUP* 4.0 BEAT. Equally weighted parsimony (MP) was used to search for optimal trees, its specific procedures and analyses were following the methods of Hofstetter et al. (2002) and Larsson & Sundberg (2011).

## Results & discussion

### Phylogenetic analysis

Six strict consensus trees were generated based on the MP analyses of the 36 ITS sequences (L = 607, CI = 0.883, RI = 0.927, RC = 0.818), shown in Plate 2 with associated bootstrap supports for branches.

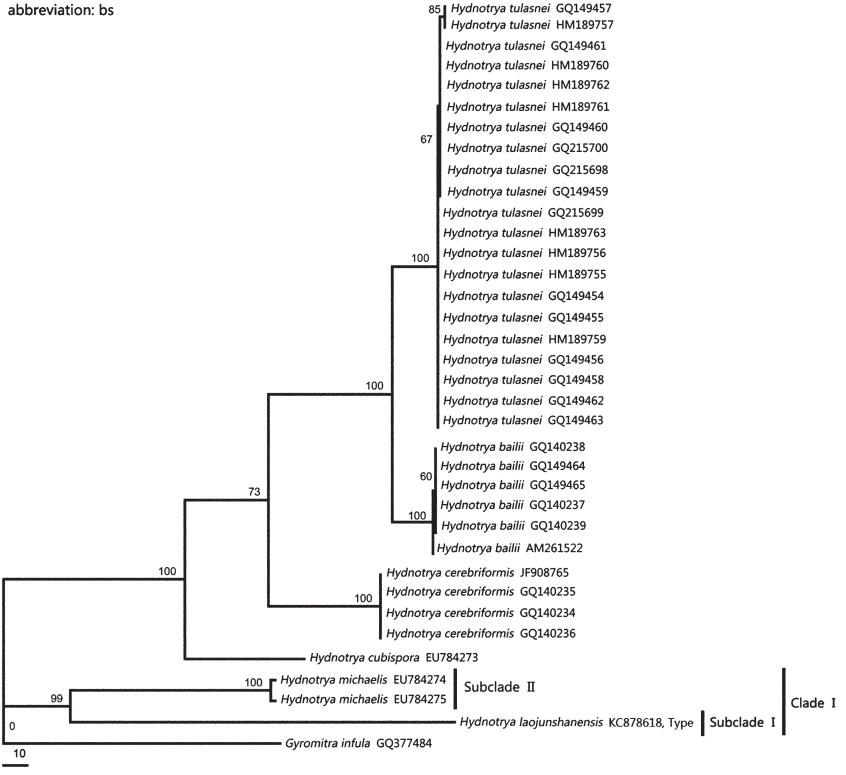


Plate 2. The strict consensus tree produced from ITS sequence analysis of *Hydnotrya* spp.

In the strict consensus tree the new species and *H. michaelis* form Clade I with 99% bootstrap support. This then subdivides into two subclades. Subclade I contains *H. laojunshanensis* and Subclade II *H. michaelis,* which has strong bootstrap support (bs = 100%). The phylogenetic analysis supports *H. laojunshanensis* as separate from (although closely related to) *H. michaelis* and distinct from other *Hydnotrya* species.

### Taxonomy

***Hydnotrya laojunshanensis*** Lin Li, D.Q. Zhou & Y.C. Zhao, **sp. nov.** Plates 3, 4

MycoBank MB 803968

Differs from all other *Hydnotrya* spp. by its single-chambered ascocarp and larger ascospores.

Type: China: Yunnan, Laojun mountains, 26°42′N 99°42′E, alt. 3786 m, in forest of *Abies forrestii* var. *smithii* Viguié & Gaussen, 30.8.2012, Lin Li (**Holotype**, YAAS L2425; GenBank KC878618).

Etymology: from the Latin for Laojun, the type locality.

Ascocarp irregularly globose, 1.0–1.2 cm in diameter when fresh, smooth, single-chambered with a primary apical opening up to 0.1–0.5 cm in diameter; cavity wall 1–2 mm thick when dry, of three layers: inner layer white, middle layer red, outer layer reddish-brown. Peridium two-layered, 605–633 μm

Plate 3. *Hydnotrya laojunshanensis* (holotype, YAAS L2425). Ascocarp.

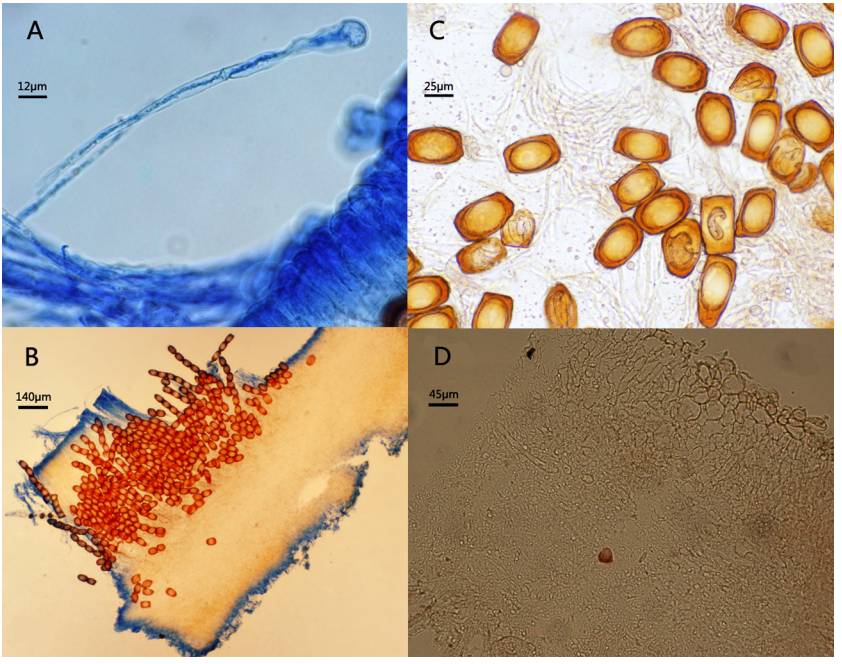


Plate 4. *Hydnotrya laojunshanensis* (holotype, YAAS L2425). A. Paraphyses in lactophenol cotton blue; B. Asci in lactophenol cotton blue; C. Ascospores in water; D. Peridium in lactophenol solution.

thick, outer layer 223–260 μm thick, pseudoparenchymatous, composed of light brown angular or irregular cells of (27.7–)33.0–46.5(–54.0) × (19.7–)31.8 (–45.0) μm, inner layer, 382–373 μm thick, consisting of hyaline interwoven hyphae. Hymenium two-layered, the one next to the peridium deep orange, another yellowish to whitish. Asci cylindrical, 342.4–401.1 × (24.2–)29.1–35.9 μm, 8-spored, regular-monoseriately arranged. Ascospore elliptical (without ornamentation) or rectangle (with ornamentation), reddish orange, (42.5–) 50.0–57.2(–60.3) × (27.5–)30.4–36.9(–38.2) μm (with ornamentation), (29.2–) 35.3–42.4(–44.0) × (20.3–)24.4–29.8(–30.0) μm (with ornamentation), Q = 1.28. Paraphyses hyaline, straight, 2.4–6 μm in diam, apical slightly inflated up to (10.8–)13.1–15.2(–18.3) μm wide, septate, protruding up to 242.6–373.0 μm.

Ecology & Distribution: solitary in the soil in an *Abies forrestii* var. *smithii* forest. Known only from Yunnan Province, China.

Comments — This species is characterized by large, rectangular ascospores and a single-chambered ascocarp, which distinguishes it from all other *Hydnotrya* species. ITS sequence analysis also shows that *H. laojunshanensis* is distinct from

other *Hydnotrya* species, although closely related to *H. michaelis*. *Hydnotrya laojunshanensis* differs in having larger ascospores and lacking any cerebriform folding, whereas *H. michaelis* typically has strongly inwardly folded lobes.

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